REVIEW ARTICLE

IN VITRO PROPAGATION OF ECONOMICALLY IMPORTANT SOME INDIAN HIMALAYAN MEDICINAL PLANT SPECIES FOR CONSERVATION AND COMMERCIALIZATION

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Abstract: Medicinal and aromatic plants form an integral and essential part of the lives of hill communities, and the inhabitants depend on these plants for their use. These plants are well known source of active principles in Ayurvedic, Unani and other traditional systems of medicines. Being source of many high value drugs, and ever increasing global demand for the "naturals", these species are being subjected to reckless, often illegal harvesting, well beyond their natural regeneration capacity. This has led to many species being listed in the Red Data Book or/in various threat categories of International Union for the Conservation of Nature and Natural Resources (IUCN). In order to face such challenges, biotechnological tools (in vitro propagation) can be used for rapid multiplication of elite clones to provide the much needed planting material for cultivation, and thus help in achieving the overall goal of conservation. The present paper deals in with the *in vitro* method being applied for some selected medicinal plants of Indian Himalayan Region (IHR).

Keywords: Conservation, Medicinal Plants, Propagation

INTRODUCTION

The medicinal and aromatic plants (MAPs) have attracted global interest as a source of natural products, and are increasingly being used as alternative to modern medicine for treatment of various diseases (Kumar and Mishra, 2011). People of all religions and culture, since ancient times to the present day; have used plants as a source of medicines. 70-80% of world population completely depend traditional, mostly herbal medicine either directly or indirectly for their primary medical assistant and needs (Farnsworth and Soejarto 1991; Pei, 2001; Patel *et. al*., 2011a & b). The greater part of traditional therapy involves the use of plant extracts or their active principles. Conservation of plants has emerged as one of the priority agenda of research and development as many species are facing the threat of extinction (Kumar & Sharma, 2011).

The Indian Himalayan Region (IHR), is over 5.3 lakh km^2 that comprises of vast mountain range extending over 2500km in length between Indus and Brahmaputra river system and rising from low lying plains to over 8000m. Wide variation in geological and geographic attributes, which causes diversity in life in the region, i.e., with about 8000 species of flowering plants (nearly 50% of total flowering plants of India) of which nearly 30% are endemic to the region, comprising over 816 tree species, 675 wild edible species and nearly 1750 medicinal plants (Samant et al., 1998). Many of these species are being used extensively as food and source of nutritive additives, fuel wood, fodder, fiber and as raw or processed high value drugs, aromatics, etc. Inhabitants of the region are traditionally well familiar with the healing properties of the available plants and therefore, their dependency on medicinal plants for primary healthcare forms integral part of their life. These plants are well known source of active principles different systems of medicines (Ayurvedic, Unani and other traditional systems of medicines).

Being the major source of high value drugs, and ever increasing global demand for the "naturals", these species are being subjected to reckless, often illegal harvesting, well beyond their natural regeneration capacity. The exploitation of important plants from the IHR has resulted in present reduction in the forest cover and simultaneously decreased the availability of many high value medicinal and economically important species. This has led to many species being listed in the Red Data Book or/in various threat categories of International Union for the Conservation of Nature and Natural resources (Samant *et al*., 1998; Ved et al., 2003).

Over the years, a total of 8321 medicinal plant species have been mentioned in the global IUCN Red List of Threatened Species. With the ever increasing human population and the requirement of plant and its products, there has been a tremendous pressure on plants, the primary producers. Therefore, in order to cope with such problems, in vitro propagation

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techniques offer great potential not only for rapid multiplication of existing stock of plant species but also for conservation of important, elite and endangered ones (Wochock, 1981; Nadeem et al., 2000; Chandra *et al*., 2006; Mukhopadhyay *et al*., 2016; Nandi *et al*., 2016).

There are a number of species which are of high commercial importance and can generate income for the locals and also bring about economic benefits. Moreover, there is a lot of variation in active ingredient content in plants among different populations and also within the same population. Keeping in mind the relevance, *in vitro* propagation methods have been applied successfully for selected high value Indian Himalayan MAPs, namely, *Aconitum balfourii*, *Podophyllum hexandrum*, *Amomum subulatum* and *Zanthoxylum armatum*. Using elite planting material, attempts have been made to establish cultures, induce multiple shoots, improve rooting, and subsequently develop suitable methods for hardening before field transfer. In selected cases genetic fidelity analysis, survival and growth of *in vitro* raised have been monitored for their performance in the field.

A brief description of *in vitro* propagation of selected medicinal plants (Fig. 6) is provided below:

Aconitum balfourii

Ayurvedic name : Vatsanabh
Unani name : Bachnak
Hindi name : Meetha vish
English name : Aconite
Local name : 'Meetha' and 'Bish'
Trade name :Meetha vish, Indian aconite

Distribution

Aconitum balfourii Stapf. (=*A. atrox* (Brhul) Muk.; family: Ranunculaceae; a high value medicinal and perennial herb. The genus Aconitum comprises of 300 species in the world with major centers of diversity in the mountains of East and South-East Asia and Central Europe (Kadota, 1987). A small group is also found in Western North America and Eastern United States of America. In Himalayan regions, it is distributed in Pakistan, India, Nepal, Bhutan, and South Tibet, where Aconitum were used in local and traditional system as medicine (Shah, 2005). In India, the genus is represented by about 24 species that is mainly distributed in sub alpine and alpine zones of Himalayas from Kashmir to Uttarakhand and extending it to the hills of Assam. Aconitum is usually found in wet alpine zone, however, in Kashmir Himalayas, these species were reported in temperate zone. Out of 300 species, a total of 33 species are found in great Himalaya (Chaudhary and Rao, 1998). *A. balfourii* Stapf is prevalent to Garhwal and Kumaon regions; it is also found in Nepal. It is generally found in the valley of flowers, Kedarnath, Tungnath, Madhyamaheshwar, and Panwalikantha on shady slopes between 3000 to 4200 m altitudes (Chopra *et al*., 1984).

Chemical Constituents

Tubers of A. balfourii Stapf mainly contain a crystalline toxic alkaloid called pseudoaconitine (0.4 to 0.5%) and aconitine in small amount (Figure 2). Three new norditerpenoid alkaloids 8-Omethylveratroylpseudaconine, veratroylbikhaconine, and balfourine have been isolated from A. balfourii Stapf together with eight known alkaloids such as: pseudaconitine, veratroylpseudaconine, indaconitine, ludaconitine, 8-deacetylyunaconitine, bikhaconitine, neoline, and chasmanine. From the aerial parts of A. balfourii Stapf are nine norditerpenoid alkaloids: condelphine, bullatine, neoline, isotalatizine, 1-Omethyldelphisine, pseudaconitine, yunaconitine, bikhaconitine, and indaconitine were isolated (Khetwal, 2007). Pseudaconitine Figure 2 is a diterpene alkaloid, with the structural formula C36H51NO12.

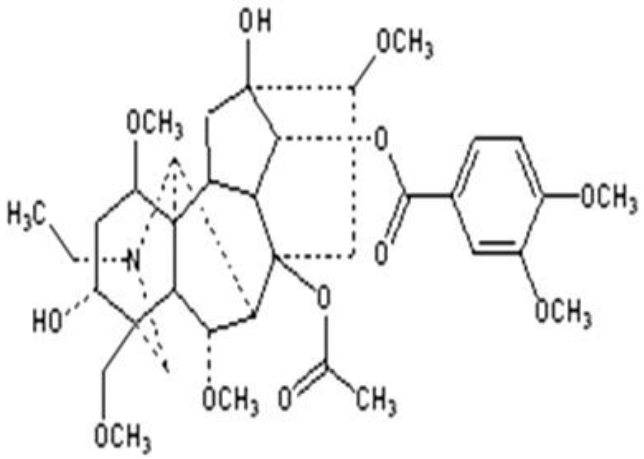

Figure 2: Chemical structure of Psudoaconitine.

The increasing demand of such naturally occurring high value compounds has resulted for indiscriminate collection from the wild habitat (Rikhari et al., 1998; Nadeem *et al*., 2000, Nandi *et al*., 2002).The present

status of this species is "Vulnerable" (Ved *et al*., 2003). These alkaloids exhibited anti-inflammatory, vermifuge, anti-rheumatic, analgesic and cardio-tonic activities and are used in different types of pains, inflammations and neuronal disorders.

Ethno-botanical uses

Alkaloids isolated from this genus exhibited anti-inflammatory, antinociceptive, hypotensive, bradycardic, analgetic, and cardiotonic activities (Ameri, 1998). The roots of Bachnak are diaphoretic, diuretic, febrifuge, anti-inflammatory, antirheumetic, antipyretic, and vermifuge. It is used in all types of pains and inflammations. In large doses, it acts as powerful sedative, narcotic and poison. The crude methanolic extracts of Aconitum species possess pharmacological activities such as antifungal, antibacterial, and insecticidal properties (Anwar *et al.*, 2003).

Tuberous roots of *A. balfourii* Stapf are rich resource of the important aconite and pseudoaconitine alkaloids. The extract is used in different system of medicines, so the commercial demand is very high. To fulfill the demand of excessive illegal collection and sale of Aconitum by farmers was continuously carried out. Other reasons are the low germination percentage and the cultivation of Aconitum species are done in a very small scale because of low availability of land for cultivation of medicinal plants (Srivastava *et al*., 2010). In addition, under natural conditions, seed germination and seedling establishment in *A. balfourii* is very rare. Growing harvestable raw material from seeds requires a lengthy cultivation cycle of 5 to 7 years (Rawat et al., 1987). Moreover, destruction of natural habitat and the earlier mentioned reasons are collectively responsible for its endangered status.

Conservation efforts

Overexploitation and habitat destructions are two major causes of threat categories of this species. Population density and degree of consistency (occurrence) was used to allocate the status of any species. The population study of three aconites, *A. balfourii*, *A. heterophyllum* and A. violaceum, was carried out in Garhwal region. In this study, the authors considered many regions in Garhwal and revealed that A. balfourii has the highest frequency (70%) and density at 3200 m (near timberline) in Dayara and the minimum (30%) in the valley of the flower. The degree of consistency of A. balfourii was frequently found at most sites and seldom in one or two pockets. They recommended that it might be due to its specific habitat requirement or due to continuous removal of plants for the medicinal uses. On the basis of their study, they have assigned *A. balfourii* and other two aconites as endangered.

Factors like long juvenile phase, poor seed germination, erratic flowering and fruiting and harsh climatic condition has resulted in diminishing status on the growth of *A. balfourii* (Pandey *et al*., 2000). Therefore, *in vitro* technique offers an alternative method of multiplication and thus source of plant propagules for species that requires periods of several years for growth and development (Bajaj, 1989; Constabel, 1990). Tubers of *A. balfourii* used as explants which were transferred on to Murashige and Skoog'smedium (MS; Murashige and Skoog, 1962) supplemented with varying concentrations (4.5, 13.5 or 22.5µM) of BAP. The axillary buds sprouted to form shoots and young leaves of these shoots were further used for initiating callus. Callus was initiated following 5 weeks on MS medium supplemented along with various combinations of BAP (0.5-4.5µM) and NAA (5.4-26.9 µM); best response (75%) for callusing was found with 4.5µM BAP and 26.9 µM NAA. Further, for shoot induction, healthy and proliferating calli were transferred on to fresh MS medium supplemented with BAP (4.5-22.2µM) and NAA (0.5-5.4 µM). It was observed that lower level of NAA (0.5-5.4µM) and BAP (4.5µM) showed excellent shoot regeneration. MS medium containing 4.5 µM BAP and 1.4 µM NAA resulted in maximum (100%) adventitious shoot formation and was routinely used for shoot multiplication. Subsequently, single shoot were separated out and sub-cultured on same MS medium containing varying concentration (0.5-44.4µM) of BAP for shoot multiplication and elongation. Out of the above mentioned combination tried, 1.1 µM BAP resulted in maximum shoot number (24.7 per flask) along with better shoot elongation (about 3.5 cm); further these shoot developed in MS medium containing 1.1 µM BAP were inoculated onto the same medium composition containing various concentration of IBA (4.9, 12.3 or 24.5 µM) for rooting, which resulted in maximum (89%) rooting on medium containing 12.3µM IBA. These plants were then transferred for hardening (in plastic pots) in green house conditions (25°C, RH 65%) for two months; following 2 months of hardening they were transferred to a mixture of soil and FYM (1:1) and allowed to grow for another 2 months before reintroduction to their natural conditions. This substantially improved the ex vitro survival (50%) and root proliferation during the period of hardening. Further, chromosome counts in *in vitro* plants and mother plants showed similar chromosome number indicating true to type regenerants (Pandey et al., 2004)

Podophyllum hexandrum

Ayurvedic Name : Giriparpat
Hindi Name : Bankakri
English Name : Mayapple
Local Name : Banwaigan' and 'Papri'
Commen Name : Himalayan May apple or Indian May apple

Distribution

Podophyllum hexandrum Royle (family: Berberidaceae) was known as (a divine drug) in ancient times. Its The perennial herb *Podophyllum hexandrum* is native to the lower elevations of

Himalayan countries like Afghanistan, Pakistan, India, Nepal, Bhutan, and in S. W. China. In India *Podophyllum hexandrum* is mostly found in Alpine Himalayas (3000-4000 msl) of Jammu and Kashmir, Himachal Pradesh, Sikkim, Uttaranchal and Arunachal Pradesh.

Chemical Constituents

Indian *Podophyllum* has a long history of usage amongst natives of the Himalayas, an aqueous extract of the roots being a common cathartic. It has also been used as a remedy in ophthalmia. Resin from the Indian plant was analyzed by Thomson in 1890, who reported 56% podophyllotoxin content. Podophyllotoxin was first shown to be the active principle of podophyllin by Podwyssotzki and was obtained in a pure state in 1880. The rhizomes of *Podophyllum hexandrum* are known to contain several lignans which are dimerisation products of phenylpropanoid pathway intermediates linked by central carbons of their side chain. It is low to the ground with glossy green, lobed leaves on its few stiff branches, and it bears a light pink flower and bright red-orange bulbous fruit. It can be propagated by seed or by dividing the rhizome. It is tolerant to cold temperatures, as would be expected of a Himalayan plant, but is not tolerant to dry conditions. *P. hexandrum*is a prominent source of podophyllotoxin which is used for the preparation of high value anti-tumour agents like etoposide, etopophos and teniposide (Figure 3) (Van Uden *et al*., 1989; Schacter, 1996; Canel *et al*., 2000) which are highly effective in lung cancer, leukaemias and other solid tumours treatment (Van Uden *et al*., 1989; Canel *et al*., 2000; Lee and Xiao, 2005).

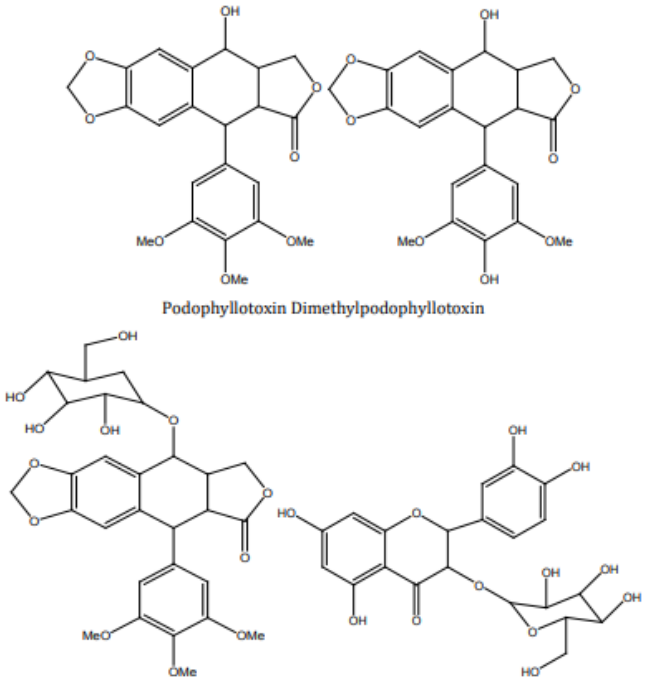

Figure 3: Chemical Compounds of *Podophyllum hexandum*

Ethno-botanical uses

In Kashmir it has been used in traditional system of medicine from time immemorial and is locally known as Banwangun, since its red colour fruit (berry) is of the size of a small brinjal. Indian Podophyllum has a long history of usage amongst natives of the Himalayas, an aqueous extract of the roots being a common cathartic. It has also been used as a remedy in ophthalmia. Resin from the Indian plant was analyzed by Thomson in 1890, who reported 56% podophyllotoxin content. Podophyllotoxin was first shown to be the active principle of podophyllin by Podwyssotzki and was obtained in a pure state in 1880. The rhizomes of *Podophyllum hexandrum* are known to contain several lignans which are dimerisation products of phenylpropanoid pathway intermediates linked by central carbons of their side chain. It is low to the ground with glossy green, lobed leaves on its few stiff branches, and it bears a light pink flower and bright red-orange bulbous fruit. It can be propagated by seed or by dividing the rhizome. It is tolerant to cold temperatures, as would be expected of a Himalayan plant, but is not tolerant to dry conditions. It has been extensively exploited in traditional Ayurvedic system of medicine for treatment of a number of ailments like Condyloma acuminata, Taenia capitis, monocytoid leukemia, Hodgkin's disease, non-Hodgkin's Lymphoma, cancer of brain, lung, bladder and venereal warts.

Conservation efforts

Seeds of *P. hexandrum* remain dormant for about 10 months under natural conditions. Strategies towards conventional and in vitro propagation of Podophyllum sp. have been tried. *In vitro* propagation is a best possible way to safeguard overexploited species and to encourage cultivation. Nadeem *et al.* (2000), successfully established a protocol for propagation of *P. hexandrum* through conventional and *in vitro* techniques). Seeds of *P. hexandrum* were collected from high altitude zone of District Bageshwar and processed for disinfection, viability testing and kept for imbibitions (overnight) as per protocol mentioned by Nadeem et al. (2000). Embryos were carefully excised from the imbibed seeds and used as explants, following inoculation on MS medium, supplemented with different concentrations of PGRs [BAP (0.5-5.0 µM); IAA (1.0-4.0 µM) or NAA (0.5-5.0µM)].Excised embryos were found to germinate within one week of inoculation with prominent cotyledonary tube possessing multiple leaves and distinct radicular portion observed following 2-3 weeks. Multiple shoot were formed when excised embryos placed on the medium containing 1.0- 4.0 µM IAA and 1.0 µM BAP. The highest shoot multiplication rate (5.0shoots/embryo) was observed on medium supplemented with 1.0 µM each of IAA and BAP. Multiple shoots were formed in the base of cotyledonary leaf of the embryos in about 4-5 weeks. Subsequently, shoots were separated and cultured separately for rooting on ½ strength medium containing 0.5 µM IAA and it resulted in rooting of 16.6% shoots (Nadeem *et al*., 2000).

Well rooted microshoots were transferred in flasks containing sterilized vermiculite and allowed to harden for 1-2 weeks under aseptic conditions. After that plantlets transferred to polybags containing vermiculite and kept in a polyhouse (25-30°C, 50% shade) for 30-35 days. All plants showed new shoot emergence and were found to behave like normal field grown plants (Nadeem *et al.* 2000).It was observed that the survival of the regenerated tissue culture raised (TCR) plants was only 30% for initial 3 months only and further there were no survival. Arumugam and Bhojwani, (1990) also reported multiplication of *P. hexandrum* using tissue culture. They multiple shoot formation from zygotic embryos only, The rooting studies of these shoots was is highly demanded but scarily available. Nadeem et al (2000) seems to be the first report on in vitro propagation of *P. hexandrum* via multiple shoot formation and also their subsequent rooting. Somatic embryogenesis followed by germination is yet another beneficial method as it mitigates the required steps of induction of root for propagation through multiple shoot formation; this has also been applied in this species (Nadeem *et al*., 2000).

P. hexandrum has a relatively long juvenile phase (Bhadula et al. 1996, Airi *et al.* 1997, Nadeem *et al.* 2000). Propagation is achieved through vegetative as well as through seed, natural regeneration is restricted by poor and erratic seed setting and trampling by grazing animals. Seeds are known to remain dormant (1-2 years), an adaptation against the harsh climate of higher altitudes. Hence, *in vitro* techniques can be an effective and other potential techniques for propagation and thus has been applied for enhancement of population of many rare and endangered plant species (Nandi *et al*., 2002, 2016; Chandra et al., 2006; Purohit *et al*., 2015).Thus, the *in vitro* protocol developed above has been found to be very effective as entire procedure beginning from culture initiation followed by plant regeneration and field hardening required 5-6 months period.

Amomum subulatum

English Name : Large cardamom
Hindi Name : Bari Elaichi

Distribution

Amomum subulatum Roxb.(family: Zingiberaceae;;) is a tall perennial rhizomatous herb and an important cash crop cultivated commercially between 600-2000 m. India being the largest producer dominates with a production of 4465 metric tons and exported 1110 metric tonin 2013-14 (Anonymous, 2015). In India, cultivation is mainly in Arunachal Pradesh, Mizoram, Manipur, Nagaland, Sikkim, West Bengal and Uttarakhand (Bisht *et al*., 2010).

Ethno-botanical uses

It is mainly used as spice. The seed has a pleasant aromatic odour due to which it is extensively used for flavoring food preparations, and has also been used in Ayurvedic medicines (Sharma et al., 2000). Seeds are also considered as an antidote to snake and scorpion venom; Traditionally, large cardamom has been used as preventive as well as a curative for throat trouble, congestion of lungs, inflammation of eyelids, digestive disorders and in the treatment of pulmonary tuberculosis. It is also useful in treatment of flatulence, loss of appetite, gastric troubles, congestion and liver complaints. The seeds are reported an official drug in Ayurvedic Pharmacopoeia which are marketed under the name of Greater Cardamom. Seeds and fruits of A. subulatum are added to cooked food items as spices by the general population on a daily basis and can play a preventive role in the occurrence of gastrointestinal disorders, respiratory problems, and through increasing palatability and flavor of foods make them more digestible and serve to maintain good health. A preparation called "Alui" is prepared for the treatment of malaria by administering the mixture of cumin (cuminium cyminum) and large cardamon (Verma *et al*., 2010; Bisht *et al*., 2011).

Chemical constituents

Plant parts of *Ammomum subulatum* mainly contain the glycosides, petunidin 3,5 – diglucoside, leucocyanidin-3-O-β-D-glucopyranoside, chalcone, cardamonin, flavanone, alpinetin and subulin. Acid hydrolysis of subulin gave the aglycone,

subulaurone. The seeds on steam distillation yield a dark brown, essential oil (2.5%) having a characteristic odour of cineol. Volatile oils present in seed containing cineol (74%), limonene (10.3%), myrcene (0.3%), α-terpinene (0.2%), 4-terpinene (0.2%). The seeds of A. subulatum contains the glycosides, Petunidin-3, 5-diglucoside, Leucocyanidin-3-O-β-D glucopyranoside, Subulin and 1- 8, Cineole, α-terpinyl acetate (Figure 4).

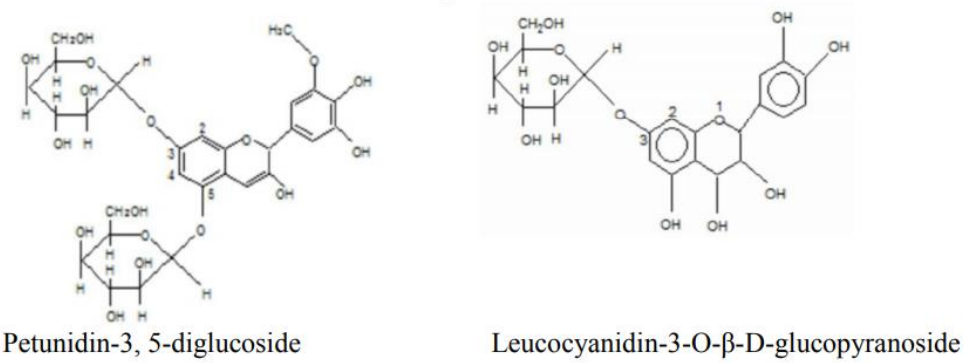

Figure 4: Chemical Compounds of *Ammomum subulatum*

Conservation efforts

In natural conditions plant propagates by seeds and rhizomes; however, very low seed germination (62%) and slower rate of plant multiplication fail to meet out the ever increasing demand of plant for expanding cultivation. Moreover, in order to cope with such problems, in vitro method of propagation provides an alternative and effective means of multiplication of such elite and high valued genotypes.

Purohit et al., 2016 using small pieces of rhizome used as explants. The scale (leaves) were cutted into 2.0-3.0 cm pieces having bud intact to it and inoculated on PGRs free MS medium. The buds (sprouted) were removed and were again inoculated in Erlenmeyer flask (250 ml) containing MS medium supplemented with various combinations of PGRs (NAA, 0.5-1.0µM; BAP, 2.0-5.0µM). Out of the various combination tried, most of the combination showed good response for shooting and rooting; however, maximum shoot (32.6) and root (61.4) per explant resulted in MS medium supplemented with 4.0 µM BAP and 1.0µM NAA. The same procedure was found to be reproducible when individually separated shoots were transferred again on the same medium.

Following this protocol on an average, about 30 plantlets could be obtained per culture cycle of three weeks. Survival percent was 90% at the end of the 5 weeks of acclimatization, and cent percent survival was recorded after 150 days of transfer into earthen pots. Random amplified polymorphic DNA (RAPD) marker analysis was performed on the regenerated plants to confirm their genetic fidelity with the mother plant and found to be true-to-type (Purohit *et al*., 2016). Thus, *in vitro* propagation protocol can be effective method for multiplication of elite and/or high yielding *A. subulatum* to meet out the ever increasing demand of planting material for expanding cultivation (Purohit et al., 2016).

Zanthoxylum armatum

Ayurvedic name : Tejovati, Tumbru (fruit)
Unani name : Kabab-e-Khanda
Hindi name : Tejbal,
English Namr : Prickly ash
Nepali Name : Dhaniya or Toothache tree
Trade name :Timru, Timur

Distribution

Zanthoxylum armatum DC; (family: Rutaceae), an important medicinal plant of IHR distributed from 1000–2100m in IHR and Eastern Ghats of India. A dioecious shrub to large tree, and characterized by sharp thorns on either the stem or foliage, is distributed worldwide from tropics to temperate regions at an altitudinal range of 1000-2100m. The medicinal properties of *Z. armatum* are well known for the treatment of stomach and tooth ache, intestinal worms, snake bites, rheumatism, scabies, fever and cholera. It is being used as deodorant, disinfectant and also possesses antiseptic properties. Most widely used part of this species is the pericarp of the fruit. Due to multiple utility of *Z. armatum* and its high cultural value for the locals, unsustainable harvest of above ground parts and low regeneration (Kala, 2010); the plant is considered under endangered category.

Ethno-botanical uses

The bark, fruits and seeds of Z. armatum are extensively used in indigenous system of medicine as a carminative, stomachic and anthelmintic. The fruit and seeds are employed as an aromatic tonic in fever and dyspepsia. An extract of the fruits is reported to be effective in expelling round worms. Because of their deodorant, disinfectant and antiseptic properties, the fruits are used in dental troubles, and their lotion for scabies. They are also used to ward-off houseflies

Chemical constituents

Various phytochemical constituents like alkaloids, sterols, phenolics, lignins, coumarins, terpenoids, flavonoids and their glycosides and benzenoids, fatty acids, alkenic acids, amino acids have been isolated from this plant. The phytochemical compounds are extracted armamide (amide), asarin and fargesin (lignan) from the bark of *Z. armatum*. Other constituents of bark are mainly of zanthonitrile and berberine (alkaloid), L-asarin, L-sesamin and L-planinin which are lignans. Volatile constituents linalool, limonene and methyl cinnamate are also found from seed. An essential oil from this plant, collected from Kashmir and Jammu, contain linalool, (64.1%), linalyl acetate, citral, geraniol methyl cinnamate, limonene and sabines1 . Leaves from Garhwal, further east in the Himalaya, yield 0.04% essential oil, containing methyl-n-nonyl ketone, 44.0; linalool, 19.5; linalyl acetate, 10.7; and sesquiterpenes, 13.0%. The structure of some of the main constituents are given in Figure 5.

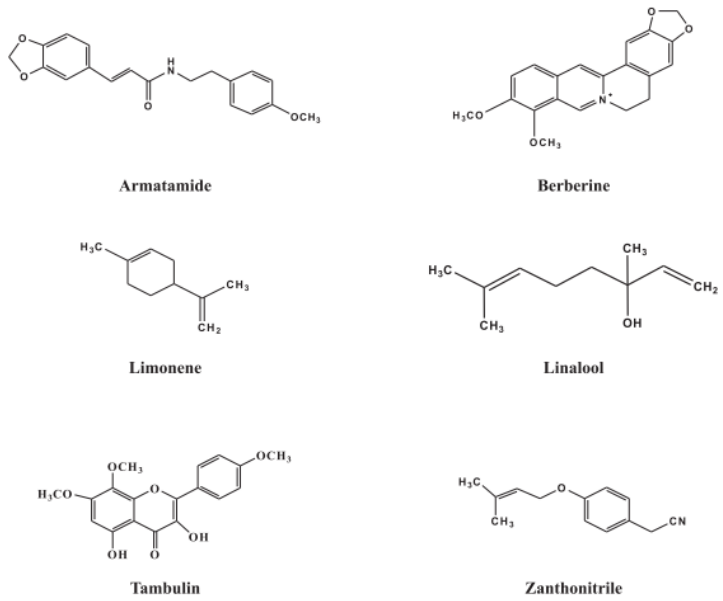

Figure 5: Chemical Compounds of *Zanthoxylum armatum*

Conservation efforts

Propagation by seed is very low (Frances, 2004). Moreover, *in vitro* propagation method offers an alternative option of propagation of such RET species. *In vitro* cultures were formed using nodal segments as explant obtained from a 2-year-old potted plant from the institute nursery at Kosi-Katarmal, Almora. MS medium supplemented with 12.0 µM BAP, 0.5 µM IAA and 0.5µM GA_3 were found to be the best possible combination for shoot proliferation (4.78 shoots/explant) and shoot length (3.46 cm). Hundred percent rooting was observed using pulse treatment of shoots on full strength MS medium supplemented with 50.0 µM IBA for 12 h, followed by subsequent transfer to ½ strength MS medium without any PGRs. Genetic fidelity assessment of the in vitro raised plants confirmed their true-to-type nature with the mother plants. The survival was found to be 75% following acclimatization. The results of this study would help in better conservation and sustainable utilization of this important species (Nandi *et al*., 2016).

CONCLUSION

The exploitation of important and valuable plants species from the IHR has resulted in decline in the forest cover and simultaneously decreased the availability of many high value medicinal and economically important species. With the ever increasing human population and the requirement of plant and its products, there has been a tremendous pressure on plants, the primary producers. Therefore, in order to cope with such problems, *in vitro* propagation techniques offer great potential not only for rapid multiplication of existing stock of plant species but also for conservation of important, elite and endangered ones. The conventional (vegetative propagation, seed germination) as well as *in vitro* techniques for propagation offers chances for 'recovery of endangered species' and also provide the scientific input to reduce the pressure from the ecosystem as well as stakeholder which are directly related to the particular or group of species, thus ultimately reducing the risk of extinction/ endangered/vulnerable of the respective species (Wochock, 1981; Nadeem *et al*., 2000; Nandi *et al*., 2002, 2016; Chandra *et al*., 2006; Purohit *et al*., 2016). In view of the importance of these high value medicinal and aromatic plants, a holistic approach for the propagation of all above discussed species, using conventional as well as *in vitro* methods needs to be adopted not only conservation but also for sustainable utilization and commercial cultivation.

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Figure 1: Selected medicinal plants growing under their natural habitat. (a) *Aconitum balfourii*, (b) *Podophyllum hexandrum*, (c)*Amomum subulatum*, and (d) *Zanthoxylum armatum*

REFERENCES

Abdi, G. and Khosh-Khui, M. (2007). Shoot regeneration via direct organogenesis from leaf segments of valerian (*Valeriana officinalis* L.). *International Journal of Agricultural Research*2: 877–882.

Airi, S., Rawal, R.S., Udhar, U. and Purohit, A.N. (1997). Population studies on *Podophyllum hexandrum* Royle - a dwindling medicinal plant of the Himalaya. *Plant Genetic Resources Newsletter*110: 29-34.

Anonymous (1988). The wealth of India. Dictionary of Indian raw material and industrial products. Raw materials, Vol. A. Publication and Information Directorate, CSIR New Delhi, India.

Anonymous (2003).Checklist of CITES species. UNEP World Conservation Monitoring Centre, Cambridge, pp 1-339.

Anonymous (2015).Annual Report 2014–15 Spices Board. Ministry of Commerce & Industry, Government of India, 137 p. http://www.indian spices.com.

Arumugam, N. and Bhojwani, S.S. (1990). Somatic embryogenesis in tissue cultures of *Podophyllum hexandrum* Royle.*Canadian Journal of Botany*68: 487–491.

Bajaj, Y.P.S. (ed.) (1989). Biotechnology in agriculture and forestry: trees II, vol 5. Springer, Berlin, 622 p

Bhadula, S.K., Singh, A., Lata, H., Kuniyal, C.P. and Purohit, A.N. (1996). Genetic resource of *Podophyllum hexandrum* Royle, an endangered medicinal species from Garhwal Himalaya, India. *Plant Genetic Resources Newsletter* 106: 26-29.

Bhandari, P., Kumar, N., Singh, B., Gupta, A.P. and Kaul, V.K. (2009). Stability indicating LC-PDA method for determination of picrosides in hepatoprotective Indian herbal preparations of *Picrorhiza kurrooa*.*Chromatographia* 69: 221-227.

Bisht, V.K., Negi, J.S., Bhandari, A.K. and Sundriyal, R.C. (2011). *Amomum subulatum* Roxb.: traditional, phytochemical and biological activities-An overview. *African Journal of Agricultural Research* 6: 5386–5390.

Bisht, V.K., Purohit, V., Negi, J.S. and Bhandari, A.K. (2010). Introduction and advancement in cultivation of large cardamom (*Amomum subulatum*Roxb.) in Uttarakhand, India. *Research Journal of Agricultural Sciences* 1: 205-208.

Chaudhary, L.B. and Rao, R.R. (1998). Notes on the genus Aconitum L.(Ranunculaceae) in north west Himalaya (India). Feddes Repertorium, 109: 527-537.

CAMP (2003). Conservation assessment and management prioritization for the medicinal plants of Himachal Pradesh, Jammu & Kashmir and Uttarakhand. In: Proceedings of the workshop held at Shimla, Hosted by Foundation for Revitalisation of local Health Traditions (FRLHT), Bangalore, India

Canel, C., Moraes, R.M., Dyan, F.E. and Ferreira, D. (2000). Molecules of interest: Podophyllotoxin. *Phytochemistry* 54: 115-120.

Chandra, B., Palni, L.M.S. and Nandi, S.K. (2006). Propagation and conservation of *Picrorhiza kurrooa* Royle ex. Benth: an endangered Himalayan medicinal herb of commercial value. *Biodiversity and Conservation* 15: 2325-2338.

Chopra, R. N., Badhwar, R. L. and Ghosh, S. (1984). Poisonous plants of India, Vol. I, Academic Publishers, Jaipur, India.

Constabel, F. (1990).Medicinal plant biotechnology. *PlantaMedica* 56: 421-426.

De Carvalho, C.M.B., Maurmann, N., Luz, D.I., Fett-Neto, A.G. and Rech, S.B. (2004). Control of development and valepotriate production by auxins in micro propagated *Valeriana glechomifolia*. *Plant Cell Reports* 23: 251-255.

Enciso-Rodriguez, R. (1997). Micropropagation of *Valeria naedulis* spp. procera. *Planta Medica* 63: 274-275.

Farnsworth, N.R. and Soejarto, D.D. (1991). Global importance of medicinal plants. In: Akerele O., Heywood V. and Synge H. (eds.). The Conservation of Medicinal Plants. Cambridge University Press, Cambridge, UK, pp. 25–51.

Frances, A. (2004). Seed storage characteristics and germination of south florida native plant seeds. Fairchild tropical botanic garden. http://www.ftg.org/PDF20Files/SeedStorageBehavior.

Jia, Q., Hong, M.F. and Minter, D. (1999). Pikuroside: a novel iridoid from *Picrorhiza kurrooa*. *Journal of Natural Products* 62: 901-903.

Kala, C.P. (2010). Assessment of availability and patterns in collection of Timroo (*Zanthoxylum armatum* DC.): a case study of Uttarakhand Himalaya. *Medicinal Plants* 2: 91-96.

Kitagawa, I., Hino, K., Nishimura T., Mukai, E., Yosioak I., Inouye H. and Yoshida T. (1969). Picroside I: a bitter principle of *Picrorhiza kurrooa*. *Tetrahedron Letters*43: 3837-3840.

Kadota, Y. (1987). A Revision of Aconitum Subgenus Aconitum (Ranunculaceae) of East Asia. Sanwa Shoyaku Co. Ltd., Utsunomiya, pp. 1-65

Kumar, A., and Mishra R.N. (2011). Computer based taxonomy in the identification of ethno-medicinal plants of Shakumbhari Devi of Shiwalik hills. *The Journal of Indian Botanical Society*, 90(3&4) 244-250.

Kumar, A. and Sharma, A. (2011). Status and conservation of some commercially exploited medicinal and aromatic plants of shakumbharidevi region of shiwalik hill. J. of Env. Bio-Sci. 25 02):269-272.

Lee, K.H. and Xiao, Z. (2005). Podophyllotoxin and analogs. In: Cragg, G.M., Kingston, D.G.I., Newman, D.J. (eds) Anticancer agents from natural products. Brunner-Routledge Psychology Press, Taylor & Francis Group, Boca Raton, pp 71–88.

Mishra, L.C. (2004). Scientific Basis for Ayurvedic Therapies.CRC Press, New York, USA.

Mukhopadhyay, M., Bantawa, P., Mondal, T.K. and Nandi, S.K. (2016). Biological and phylogenetic advancements of *Gaultheria fragrantissima*: Economically important oil bearing medicinal plant. *Industrial Crops and Products* 81:91-99.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiologia Plantarum* 15:473-497.

Nadeem, M., Palni, L.M.S., Purohit, A.N., Pandey, H. and Nandi, S.K. (2000). Propagation and conservation of *Podophyllum hexandrum* Royle: an important medicinal herb. *Biological Conservation* 92: 121-129.

Nandi, S.K., Palni, L.M.S. and Kumar, A. (Eds.). (2002). Role of Plant Tissue Culture in Biodiversity Conservation and Economic Development. Himvikas Occasional Publication No. 15.GyanodayaPrakashan, Nainital, p. 646, ISBN: 81-85097-55-0.

Nandi, S.K., Palni, L.M.S., Pandey, H., Chandra, B. and Nadeem, M. (2016). Selection of Elites and *in vitro* propagation of selected high-value Himalayan medicinal herbs for sustainable utilization and conservation. In *Plant Tissue Culture: Propagation, Conservation and Crop Improvement*, pp. 15-44; Springer Singapore. (DOI 10.1007/978-981-10-1917-3_2).

Pandey, H., Nandi, S.K., Kumar, A., Palni, U.T., Chandra, B. and Palni, L.M.S. (2004). *In vitro* propagation of *Aconitum balfourii* Stapf.: an important aconite of the Himalayan alpines. *Journal of Horticultural Science and Biotechnology* 79: 34-41.

Pandey, H., Nandi, S.K., Nadeem, M. and Palni, L.M.S. (2000). Chemical stimulation of seed germination in *Aconitum heterophyllum* Wall.and *A. balfourii* Stapf.: important Himalayan species of medicinal value. *Seed Science and Technology* 28: 39-48.

Patel, P.K., Kumar, A., Sharma, A. and Dhiman, M. (2011a). Ethnomedicinal survey of the Rishikesh and neighbouring area.J. Env. Bio-Sci., 25 (2):241-246

Patel, P.K., Kumar, A., Sharma, A. and Dhiman, M. (2011b). Traditional knowledge on medicinal plants used by van gujjar of shakumbharidevi of shiwalik hills. Plant Archives 11 (2): 587-592.

Pei, S. (2001). Ethnobotanical approaches of traditional medicine studies: some experiences from Asia. *Pharmaceutical Biology*39: 74-79.

Prakash, V. (1999). Indian Valerianceae: A Monograph on Medicinally Important Family. Scientific Publishers, Jodhpur, India, 70 p.

Purohit, S., Nandi, S.K., Paul, S., Tariq, M. and Palni, L.M.S. (2016). Micropropagation and genetic fidelity analysis in *Amomum subulatum* Roxb. *Journal of Applied Research on Medicinal and Aromatic Plants* DOI:10.1016/j.jarmap.2016.07.003

Purohit, S., Rawat, V., Jugran, A.K., Singh, R.V., Bhatt, I.D. and Nandi, S.K. (2015). Micro propagation and genetic fidelity analysis in *Valeriana jatamansi* Jones. *Journal of Applied Research on Medicinal and Aromatic Plants*2: 15-20.

Rastogi, R.P., Sharma, V.N. and Siddiqui, S. (1949). Chemical examination of *Picrorhiza kurrooa* Benth. *Indian J.Sci. Ind. Res*8B: 172.

Reza, A.G., Morteza, K.K. and Akhtar, S. (2009). Rapid micro propagation through shoot regeneration of *Valeriana officinalis* L. *Horticulture Environment and Biotechnology*5 0: 467-471.

Rikhari, H. C., Palni, L. M. S., Sharma, S. and Nandi, S. K. (1998). Himalayan yew: Stand structure, canopy damage, regeneration and conservation strategy. *Environmental Conservation* 25: 334-341.

Salles, L.A., Silva, A.L., Fett-Neto, A.G., Von Poser, G.L. and Rech, S.B. (2002). *Valeriana glechomifolia*: *in vitro* propagation and production of valepotriates. *Plant Science* 1 63:165-168.

Samant, S. S., Dhar, U. and Palni, L. M. S. (1998). Medicinal plants of Indian Himalaya: Diversity, distribution and potential values. Himavikas Publication, GyanodayaPrakashan, Nainital, India.

Schacter, L. (1996). Etoposide phosphate: what, why, where and how? *Seminars in Oncology* 23:1-7.

Shah, N.C. (2005). Conservation aspect of Aconitum species in the Himalayas with special reference to Uttaranchal India. Med. Plant Conserv., 11: 9-15. http://cmsdata.iucn.org/downloads/mpc11.pdf

Sharma, E., Sharma, R. and Singh, K.K. (2000). A boon for mountain populations: large cardamom farming in the Sikkim Himalaya. *Mountain Research and Development*20:108-111.

Singh, N., Gupta, A.P., Singh, B. and Kaul, V.K. (2006). Quantification of valeric acid in *Valeriana jatamansi* and *Valeriana officinalis* by HPTLC. *Chromatographia* 63:209-213.

Smit, H.F., Berg, A.J.J., van den Kroes, B.H., Beukelman, C.J., Ufford, H.C., Quarles, V., Dijk, H.V. and Labadie, R.P. (2000). Inhibition of T-lymphocyte proliferation by cucurbitacins from *P. scrophula riiflora*. *Journal of Natural Products* 63: 1300-1302.

Sturm, S. and Stuppner, H. (2000). Analysis of cucurbitacins in medicinal plants by high pressure liquid chromatography-mass spectrometry.*Phytochemical Analysis* 11: 121-127.

Thakur, R. S., Puri, H.S. and Hussain A. (1989). Major Medicinal Plants of India. CIMAP, Lucknow, pp.404-407

Van Uden, W., Pras, N., Visser, J.F. and Malingre, T.M. (1989). Detection and identification of podophyllotoxin produced by cell cultures derived from *Podophyllum hexandrum* Royle. *Plant Cell Reports* 8: 165-168.

Ved, D.K., Kinhal, G.A., Ravikumar, K., Prabhakaran, V., Ghate, U., Vijaya, S.R. and Indresha, J.H. (2003). Conservation assessment and management prioritization for the medicinal plants of Himachal Pradesh, Jammu & Kashmir and Uttaranchal. Foundation of Revitalization of Local Health Traditions (FRLHT), Bangalore, pp 1-24

Verma, S.K., Rajeevan, V., Bordia, A. and Jain, V. (2010). Greater cardamom (*Amomum subulatum* Roxb.), a cardio-adaptogen against physical stress. *Journal of Herbal Medicine and Toxicology* 4: 55-58.

Violon, C, Van, C.N. and Vercruysse, A. (1983). Valepotriate content in different in vitro cultures of Valerianaceae.*PharmaceutischWeekblad* 5: 205-209.

Winges, K., Kloss, P. and Henkels, W.D. (1972). Natural products from medicinal plants. XVII, picroside II, a new 6-vanilloyl catapol from *Picrorhiza kurrooa*. *LeibigsAnnalenChem* 759: 173-182.

Wochock, Z.S. (1981). The role of tissue culture in preserving threatened and endangered plant species. *Biological Conservation*20: 83-89.